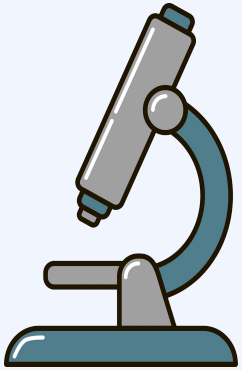


IAL BIOLOGY REVISION UNIT 3

N. Ali

Core practicals

- CP 01 - Benedict's test for reducing sugars
- CP 02 - Vitamin C content of food and drink
- CP 03 - Investigating membrane properties
- CP 04 - Factors affecting enzyme reactions
- CP 05 - Using a light microscope
- CP 06 - Stages of mitosis
- CP 07 - Observe and draw plant structures
- CP 08 - Tensile strength plant fibres
- CP 09 - Antimicrobial properties of plants

General info

Variables

Independent variable : factor changed in the investigation.

Dependent variable : measured variable that changes as a result of independent variable.

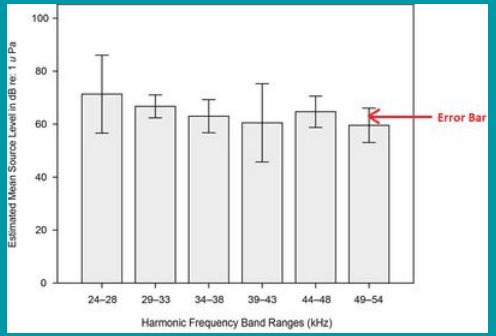
Controlled variables : factors that must be constant to ensure a valid experiment.

Control experiments : experiment with the independent variable removed to ensure it is the one causing change in dependent variable
Eg. An experiment on antimicrobial properties of extracts with wells in agar jelly is done, one well is filled with water / solvent instead of the extract with active ingredient.

General info

Standard deviation: a measure of spread of data around the mean.
Represented on graphs as error bars. Given as ± in tables.

There is no significant difference if the error bars overlap.
Validity is also affected.
If error bars are large then the data is not reliable.



- **Standard deviation formula:**

$$\sigma = \sqrt{\frac{\sum(x-\mu)^2}{n-1}}$$

  - Where:
    - **σ** stands for standard deviation
    - **x** stands for each value in the data set
    - **μ** stands for the mean of the data set
    - **N** stands for the number of values in the data set

General info

Statistical tests :
important for scientists to determine whether differences between data sets are significant or if it is due to natural variation (due to chance)

Chi squared
used to see if the results gathered during an experiment (the observed results) are what we expected to find (the expected results). We first have to state the null hypothesis - the null hypothesis is always that there is no significant difference between the observed and expected results. The chi squared test will allow us to either accept or reject the null hypothesis.
The critical value is the value of chi squared that corresponds to a 0.05 (5%) level of probability that the difference between our observed and expected results is due to chance. If your chi squared value is larger than or equal to the critical value then there is a significant difference between the observed and expected results. This means that something other than chance is causing the difference in the results and the null hypothesis can be rejected.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

General info

The Student's t-test is used to determine whether the means for two sets of data are significantly different.
The degrees of freedom is calculated by doing (n1 + n2) - 2
if the value obtained from the t-test is greater than the critical value at a probability of 5% then there is a 95 percentage chance that the difference between the 2 means is significant and not due to chance

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{(S_1^2/n_1) + (S_2^2/n_2)}}$$

$\bar{x}$ = MEAN

s = STANDARD DEVIATION

n = NUMBER OF VALUES IN EACH GROUP

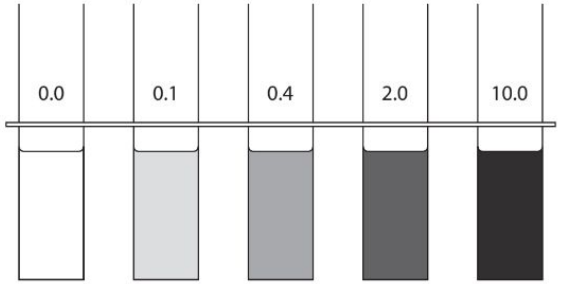
General info

Explain what is meant by the term 'valid results'. (3marks)

- Valid results measure what they are supposed to. (1)
- The measurements that have been made are affected by a single independent variable . (1)
- There is no observer bias (1)
- All variables apart from the independent variable have been controlled. / All variables affecting results other than independent variable are kept constant. (1)

To achieve reliability …repeat experiment , calculate mean and calculate SD+ large sample size

(iii) Protein solutions of concentrations from 0.0 to 10.0 mg cm^{-3} were tested using a semi-quantitative method.
The results of the test are shown in the diagram.



Describe the difference between a semi-quantitative and a quantitative test. (2)

Semiquantitative is a Rough estimation to the concentration of a substance present comparing it against standard / known concentration

Which involves : subjective judgement to color change

Quantitative method : Exact determination to the concentration of substance present .

Measure the intensity of color using a colorimeter and using calibration curve..

(iv) Explain how the results shown in the diagram can be used to compare the protein concentrations of food extracts. (2)

1 Get the sample and add biuret reagent

1 Compare the color produced against the tubes shown in the diagram

Where the concentration is estimated by deciding which protein solution in th diagram has the closet color to the tested food sample

1 Where the darker the color the higher the concentration of protein .

1 An investigation compared the protein content of some foods.

Each food was crushed in distilled water to produce a suspension. This was filtered and the liquid filtrate was tested for protein.

(a) (i) Describe how you could test for protein in this filtrate.

(2)

(1) Add biuret reagent
To the sample
(1) And if color turns from blue to purple color then protein is present

(ii) Describe how the filtrates should have been prepared to allow a valid comparison of the protein content of these foods.

Controlled variables

(3)

Same mass of food sample
Same volume of water used for extraction
Same temperature
Same type and size of filter paper

Dilution plating

To calculate the volume of stock solution needed to dilute it to the right concentration the following equation is used :

- $m_1 \times v_1 = m_2 \times v_2$
  - *m* might be commonly written as *c* too.
  - m_1 stands for the concentration of the stock solution
  - v_1 stands for the volume of stock solution required
  - m_2 stands for the concentration needed at the end
  - v_1 stands for the volume of the diluted solution.

5. When the reactivity of an enzyme is unknown, a pilot study using serial dilutions may be carried out.

(**a**) Explain the term 'serial dilution' (2marks).

A stepwise dilution of a solution (1) The dilution factor at each step is constant / the concentration decreases by the same factor with each step. (1)

(**b**) Explain the advantages of using serial dilutions. (3 marks)

The dilutions cover a wide range (and are therefore good for pilot studies). (1)Dilutions are easy to carry out as the steps are similar in each case. (1) Dilutions cover the range evenly.. (1)

(**c**) . Describe how you would carry out a serial dilution of the 1% trypsin stock solution to make concentrations of 0.1%, 0.01% and 0.001%. You may use a labelled diagram to help with your explanation. (5 marks)

Mix 1cm3 of the 1% stock with 9cm3 of distilled water. (1) This produces a 10-fold dilution / a 0.1% solution. (1) Then mix 1cm3 of the 0.1% solution with 9cm3 of distilled water to make the 0.01% solution. (1) Then mix 1cm3 of the 0.01% solution with 9cm3 of distilled water to make the 0.001% solution. (1) Mention of suitable equipment for measuring volumes, e.g. pipette. (1) Allow other suitable volumes that would give dilutions by a factor of 10. Marks can be awarded for a diagram.

(**d**) What type of scale would you use when plotting these concentrations on a graph?

A logarithmic scale (1)

Cp 1

Use a semi-quantitative method with Benedict's reagent to estimate the concentrations of reducing sugars and with iodine solution to estimate the concentrations of starch, using colour standards.

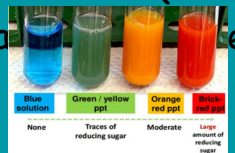
Benedict's test for reducing sugars

Variables: Independent variable: The concentration of the reducing sugar solutions Dependent variable: The colour of the solution after the addition of Benedict's reagent

Equipment: Laboratory safety wear: Lab coats, eye goggles, latex gloves. Test tubes, test tube rack, tongs Water bath at 80°C Timer Benedict's reagent Distilled water Stock solution of a reducing sugar

Procedure: Plan various concentrations of a reducing sugar from a stock solution with the help of dilutions planning up to 10 cm^3 each. Pipette only 5 cm^3 of the solutions into a separate test tube. Add 2 cm^3 of Benedict's reagent into the test tubes. Place all of the test tubes gently and carefully into the water bath. Start a timer for 2 minutes. After 2 minutes, carefully remove the test tubes with the help of tongs and place them on a test tube rack. Observe the colour of the solution in each test tube.

Observations and analysis: Precipitate is formed when Benedict's reagent as it contains copper ions which gets reduced from Cu^{2+} to Cu^{+}, causing the colour change. The colours of the solution can be compared to an existing colour scale from blue (which contains no reducing sugar at all) to brick red (which contains a very high concentration of reducing sugars) in order to estimate the concentra educing sugars in the original solution.



Cp 1

Iodine test for starch
Variables: Independent variable: The concentration of the starch solutions
Dependent variable: The colour of the solution after the addition of the Iodine solution
Equipment: Laboratory safety wear: Lab coats, eye goggles, latex gloves. Test tubes, test tube rack, tongs Iodine solution Distilled water Stock solution of starch
Procedure: Plan various concentrations of starch from a stock solution with the help of dilutions planning up to 10 cm^3 each. Pipette only 5 cm^3 of the solutions into a separate test tube. Add 2 cm^3 of Iodine solution into the test tubes. Observe the colour of the solution in each test tube. Observations and analysis: Negative result: Brown colour (No starch present) Positi
Blue colour (Moderate amount present) to Black colour (High con
present)

Risk assessment

Hazard	Risk	Precaution
Liquids	Spillage that could cause surfaces to be slippery leading to an accident	Wipe up any liquid spillages as soon as they occur Put lids on bottles Put bottles away once used Keep away from edge of desk
Hot water bath	Scalding	Take care in removing and replacing the water bath lid and have a first aid kit nearby Remove test tubes from the water bath with tongs Keep away from edge of desk
Glassware	Cuts from sharp objects	Take care when handling glass objects Keep away from edge of desk

(b) (i) Explain how a 0.4 mol dm^{-3} solution could be made from a 1.0 mol dm^{-3} sucrose solution.

(2)

Question Number	Answer
3(b)(i)	An answer including the following points: • the liquids should be mixed in ratio 2:3 (1) • The 3 is water (1)

3 The protein content of food can be estimated using a semi-quantitative test.

(a) (i) Name a reagent that can produce a semi-quantitative test for protein.

(1)

.....

(ii) State the colour change that occurs when this reagent is added to a protein solution.

(1)

Biuret	Accept copper sulfate and sodium hydroxide (sodium potassium tartrate)

Answer	Additional Guidance
Blue to {mauve / lilac / purple}	

Cp 1 dilution plating

(d) A student repeated the result for the 2% protease solution.

Determine how 1 cm^3 of a 2% solution could be obtained from a 5% stock solution of protease.

(2)

3(d)	An answer including the following points: • Dilution factor calculated (1) • Volume of 5% solution and water stated (1)	e.g. 5 ÷ 2= 2.5 accept $C_1V_1 = C_2V_2$ accept 4 stock to 6 water e.g. 1 cm^3 of 5% added to 1.5 cm^3 of water 0.4 cm^3 of 5% to 0.6 cm^3 water 2 cm^3 to 3 cm^3 of water correct answer with no working gains 2 marks

2 A simple test can be used to assess the quantity of sugar in liquids, such as food extracts and urine.

(a) (i) Describe how a urine sample can be tested for glucose.

(2)

- addition of (Benedict's / Fehling's) solution (1)
- heat / place in water bath (and observe colour change) (1)

Cp 1 food tests, serial dilution

Q1. Oct/Nov 2021

Q2. May/June 2019

Q3. May/June 2022

Q3. Oct/Nov 2021 +rate rxn

Q3. Jan 2022

Q2. Oct/Nov 2023 (gluc)

Q3. Jan 2024 (protein)

Cp 2

vitamin C content of food and drink

Equipment list

1% DCPIP solution , 5 cm^3 syringes, Test tubes , Test tube rack, Pipettes, 4 different fruit juices, 1% vitamin C solution

Method

1. Use a pipette to transfer 1 cm^3 of DCPIP solution to a test tube.
2. Then fill a 5 cm^3 syringe with 5 cm^3 of vitamin C solution.
3. Add the vitamin C solution from the syringe one drop at a time to the test tube containing the DCPIP, being sure to gently shake the test tube after each addition.
4. Stop adding the vitamin C once the blue DCPIP has turned colourless. Record the volume of the solution that has been added to cause the colour change.
5. Repeat steps 1-4 to obtain 2 further results for the vitamin C solution and then calculate a mean volume added, first discarding any anomalous results.
6. Then repeat steps 1-5 using each of the 4 fruit juices in place of the vitamin C solution.

Cp 2

Observations and analysis:
The DCPIP solution is blue in colour. Vitamin C is an antioxidant. The vitamin C found in fruit juices can be used to reduce the DCPIP solution, causing the decolourisation. 1 cm^3 of the vitamin C solution contains 10 mg of vitamin C. With this, it is possible to calculate the concentration of Vitamin C required to decolourise a solution of DCPIP by using the following formula:

$$\text{Concentration} = \frac{\text{mean volume of standard solution}}{\text{mean volume of fruit juice}} \times \text{concentration of standard solution}$$

Example

conc. of fruit juice 1 = $\frac{1.4}{2.0} \times 1 = 0.7\ \%$ concentration

1.4: volume of vitamin C solution
1: concentration of vitamin C solution
2.0: volume of fruit juice needed to decolorise DCPIP

Safety: use gloves and goggles. Avoid DCPIP contact with skin, can irritate skin. always
transfer it with a pipette or syringe
Conclusion: When vitamin C is added to DCPIP the DCPIP is reduced which eventually causes a colour change from blue to colourless. Since the volume and concentration of both the vitamin C solution and DCPIP are known, the concentration of vitamin C in each fruit juice can be calculated by comparing it to the standard solution.

Cp 2 vit c

(b) The effect of storage time and temperature on the vitamin C content of four foods was investigated.

(i) Describe a method to allow a quantitative comparison of the vitamin C content of two of these foods.

(5)

Question Number	Answer	Additional Guidance	Mark
1(b)(i)	A description that includes the following points: • use {equal / controlled} masses of the foods (1) • use {standard / same} extraction method (1) • measure out {equal / same} volume of DCPIP solution (1) • add food extract dropwise until {no colour remains / blue to colourless} (1) • record {volume / number of drops} used (1) • explanation of how to calculate vitamin C content / description of calibration of DCPIP with known vitamin C concentration (1)	Accept stated detail e.g. volume of distilled water / time of macerating / standard filtering method measure out {equal / same} food extract Add DCPIP solution dropwise until stays blue / colour stays / no change e.g. conc. vitamin C = vol. of standard Soln. vit C ÷ volume of food extract added Ignore colorimeter, colour charts	Expert (5)

www.dynamicpa

Cp 2 vit c
Q1. Oct/Nov 2022
Q1. Jan 2024

Cp 3

Beetroot cells have betalain pigments that leaks out when the cell membrane is damaged as it is normally too big to pass through

Equipment : Beetroot , Scalpel , 4 test tubes , Colorimeter ,Cuvettes , Stopwatch , Water baths, Pipette

Method

1. Use the scalpel to cut 4 equal pieces of beetroot from the same beetroot and rinse each piece with water to remove any pigment already released from the cutting.
2. Use a pipette to fill 4 test tubes with 5 cm^3 of water and place a cube of beetroot in each test tube.
3. Place each of the test tubes in a water bath at the following temperatures - 0°C, 20°C, 40° C, and 60°C. Remove the test tubes from the water baths after exactly 5 minutes and remove the beetroot pieces leaving only the coloured liquid.
4. Set up a colorimeter by giving it 5 minutes to stabilise and by first measuring its absorbance with pure water in a cuvette. Then use a pipette to fill a cuvette with liquid from each of the 4 test tubes to measure and record their absorbance with the colorimeter. 5. Plot a graph of temperature against absorbance.

Cp 3

Hazard : ethanol highly flammable. Keep away from naked flames and extreme heat Put the lid on once used.

Conclusion : Temperature

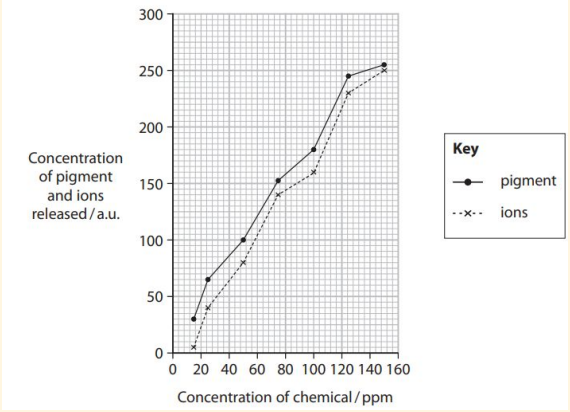
- Below 0 °C - Ice crystals form in the membrane, piercing it and allowing molecules including beetroot pigment to leak out.
- 0°C - 40°C - As temperature increases the phospholipids in the membrane gain kinetic energy and move more, increasing the permeability.
- Over 40°C - Proteins in the membrane deform at high temperatures meaning they cannot control what goes in and out of the cell - increase membrane permeability. Furthermore, the phospholipids start to melt and expanding water inside the cell puts increasing pressure on the membrane.

Alcohol

Both ethanol and the phospholipid bilayer that makes up the cell membrane are non-polar. This means the lipids dissolve in solutions containing ethanol, thus disrupting the membrane. The higher the concentration of lipids the more the membrane will be disrupted allowing more pigment to leak out.

(iii) Devise a method that could be used to obtain the data for the pigment shown in the graph.

(5)

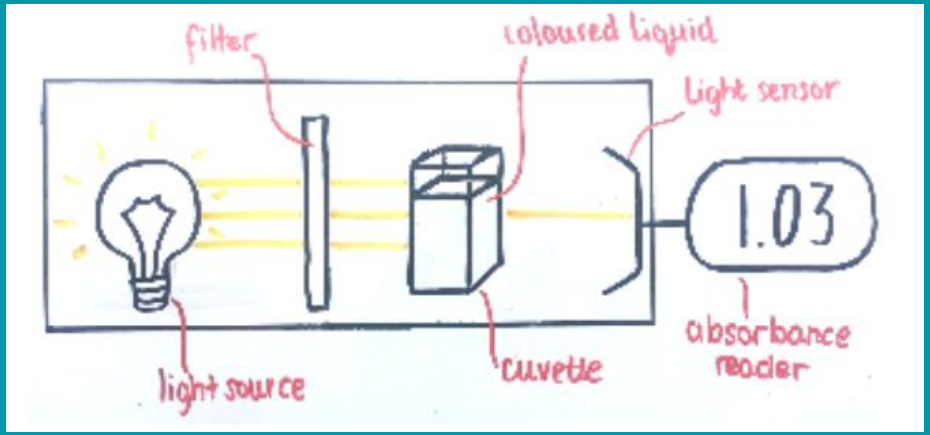


An answer that includes the following points:	
• equal sized pieces of plant tissue cut (1)	Accept alternatives e.g.mass
• washed (in distilled water until no more pigment lost) (1)	Accept rinsed
• placed in a range of 7 concentrations of the chemical shown (1)	Allow 8 if it is clear one is distilled water or 0%
• samples of the liquid (around the discs) were removed / tissue removed from tube (1)	
• method for measurement of intensity of colour described (1)	colorimeter (or implied), looking down tubes, compare with colour standard
• one variable identified	e.g. temperature, pH, time in water, volume of solution

Cp 3 cell membrane
Q1. May/June 2020
Q1. Jan 2018
Q3. Jan 2020
Q2. Jan 2023
Q3. Jan 2023
Q3. Jan 2022
Q2. Jan 2024

Cp 4

Four factors are being investigated in this enzyme controlled experiments: Temperature, pH, enzyme and substrate concentration. Milk is used as it contains a protein called casein that gets digested by enzymes like trypsin. Milk is white and opaque and as the protein is digested, the solution gets more translucent which allows for light to pass through more easily. A colorimeter is used to see how much light has passed through the solution, so that we can measure



Cp 4

effect of different temperatures on rate of enzyme catalysed reaction

1. Carry out the experiment under 5 different temperatures (10,20 ', 30,40,50 C) and control the temperature using thermostatic water bath .
2. Get 5 test tubes add equal volume of (2Cm3) of 1 % trypsin
3. Get another 5 test tubes and add equal volume of milk
4. Allow equilibration (leaving test tubes for 5 mins at exp temp)
5. Control pH using buffer solution
6. Set the colorimeter to zero absorbance …by getting a blank cuvette filled with water and trypsin .
7. Mix the trypsin with milk …shaking gently …..then place the solution in the cuvette to be put in the colorimeter …read the absorbance of light

Repeat with the other 4 Test tubes at other temperatures Remember to use the reference cuvette to set the colorimeter to zero absorbance

Results: record time (s) against absorbance (au)

Cp 4

Determining initial rate of reaction from a graph:
A tangent is drawn where the graph is at the start of the reaction.
Two points on the tangent are selected and placed into the gradient formula to find the initial rate of reaction.

- Gradient = $\frac{\text{Change in y}}{\text{Change in x}} = \frac{y_2 - y_1}{x_2 - x_1}$

Cp 4

Explain why it is important to measure the initial rate of the reaction rather than an average rate over a longer time period. ?
Because the reaction is rapid and the milk (substrate) concentration quickly declines. The rate slows as the substrate is used up. Therefore, it is only possible to make valid comparisons at the start of the reaction, when controlled variables such as substrate concentration are the same for all levels of the independent variable.

If the surface of the cuvette is scratched, this can result in a greater absorbance of light. If the cuvette used for the reaction was scratched (but the reference cuvette was not), would this give a random or a systematic error? Explain your answer.
• A systematic error, because it would cause absorbance readings to be higher than the true value for every measurement.

Lipid breakdown experiment : why is buffer used?
To control the pH As pH would change by using the fatty acids Which would cause denature of enzymes Changing the shape of their active site By changing the hydrogen ion concentration thus affecting the charges of the R groups of amino acids at the active site. Thus changing the rate of reaction

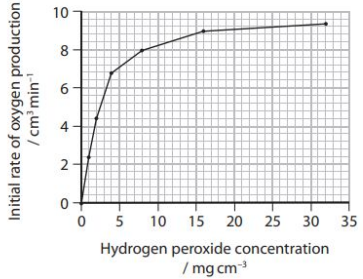
7 Enzymes are involved in many chemical reactions.

(a) State what is meant by the term **enzyme**.

(1)

(b) Catalase is an enzyme that breaks down hydrogen peroxide into water and oxygen.

The graph below shows the effect of catalase on the initial rate of reaction at different concentrations of hydrogen peroxide.



(i) Explain why it is necessary to measure the initial rate of reaction when investigating the effect of substrate concentration on enzyme activity.

(2)

Number			
7(a)	1. biological catalyst ;	**ACCEPT** {protein / chemical} catalyst {protein / molecule / chemical} that {lowers the activation energy / speeds up reactions} **IGNORE-**substance	**(1)**

Question Number	Answer	Additional Guidance	Mark
7(b)(i)	1. (as the reaction proceeds) the {substrate / hydrogen peroxide} is used up /decreases in concentration ; 2. (therefore) {substrate / hydrogen peroxide} limits the rate of reaction / {substrate / hydrogen peroxide} becomes the limiting factor ;		**(2)**

(ii) Using the graph, explain the effect of substrate concentration on the activity of catalase.

(3)

7(b)(ii)	1. as {substrate / hydrogen peroxide} concentration increases {activity of catalase / rate of reaction / oxygen production} increases / eq ;	**1 ACCEPT** positive correlation **1 DO NOT ACCEPT**-linear/proportional
	2. because more {substrate / hydrogen peroxide} to collide with {active site / enzymes} ;	**2 ACCEPT** more enzyme substrate complexes formed
	3. idea that {catalase activity / rate of reaction / oxygen production} {levels off / plateaus / eq} as {substrate / hydrogen peroxide} increases ;	**3 DO NOT ACCEPT**-rate decreases/slows down/becomes constant
	4. (as) {enzyme / catalase} concentration becomes limiting / no more active sites are available / eq ;	**4 ACCEPT** enzyme concentration becomes the limiting factor

*(c) Describe an experiment that could be carried out to collect data to plot a graph similar to the one shown in part (b).

(5)

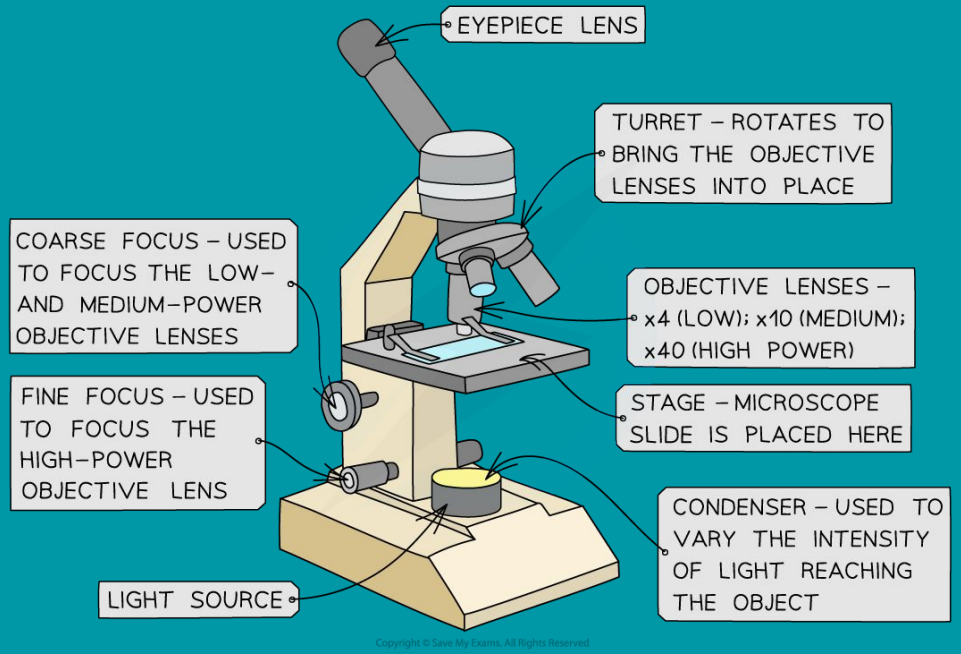
Number			
***7(c)**	**QWC emphasis is clarity of expression** 1. idea of using different concentration of {substrate / hydrogen peroxide} ; 2. description of how to measure oxygen ; 3. idea of collecting oxygen over a period of time ; 4. {measure / calculate / eq} initial rate of reaction ; 5. repeat (each concentration of substrate) and calculate a {mean / average} ; 6. two controlled variables identified ; 7. description of how to control one named variable ;	**QWC emphasis is clarity of expression** **NB candidates who describe a wrong experiment could possibly be awarded mp 5, 6 and 7** **DO NOT ACCEPT** less than 5 stated concentrations **2 e.g.** gas syringe, measuring cylinder, counting bubbles **7 e.g.** temperature using a water bath / pH using a buffer	**(5)**

Cp 4 enzyme controlled rxns
Q1. Jan 2015
Q1. May/June 2018
Q1. Maj/June 2019
Q2. May/June 2020
Q2. May/June 2022

Cp 5

Microscopes are used to understand the functioning of cells and their organelles.

The microscope you typically find in school is a light microscope. light microscopes can barely make out organelles as they have a low magnification - the degree which an imagine of an object is enlarged- and low resolution- degree which it is possible to distinguish between 2 points that are close together.


EYEPIECE LENS
TURRET – ROTATES TO BRING THE OBJECTIVE LENSES INTO PLACE
COARSE FOCUS – USED TO FOCUS THE LOW– AND MEDIUM–POWER OBJECTIVE LENSES
OBJECTIVE LENSES – x4 (LOW); x10 (MEDIUM); x40 (HIGH POWER)
FINE FOCUS – USED TO FOCUS THE HIGH–POWER OBJECTIVE LENS
STAGE – MICROSCOPE SLIDE IS PLACED HERE
CONDENSER – USED TO VARY THE INTENSITY OF LIGHT REACHING THE OBJECT
LIGHT SOURCE
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Cp 5

Electron microscopes are used by scientists to observe organelles of cells.

There are 2 types of electronic microscopes a scanning electron microscope SEM and a transmission electron microscope TEM

Stains and dyes are applied to tissue samples and bind to organelles making them easier to view and also increases contrast in image formed making it easier to see 2 objects apart increasing resolution.

	scanning electron microscope	transmission electron microscope
Image nature	3D	2D
magnification	Maximum of 2 million magnification	Maximum of 50 million magnification
Resolution	Lower resolution	Higher resolution

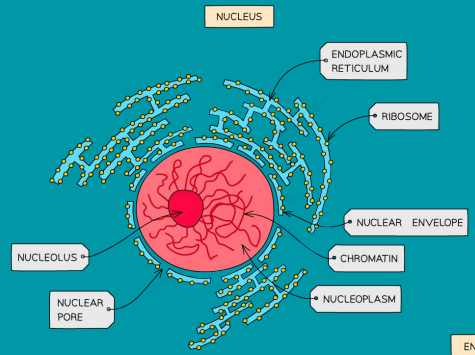
Cp 5

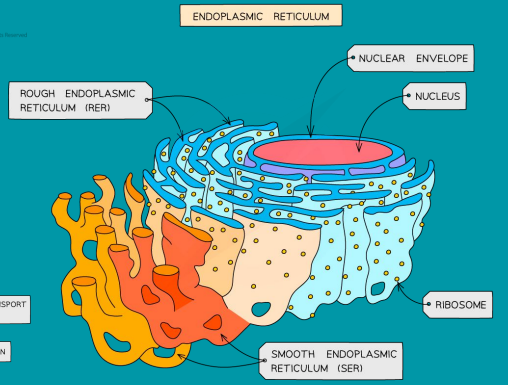
- **Nucleus**-surrounded by an envelope/double membrane containing pores which enable molecules to enter and leave the nucleus. Also contains chromatin and a nucleolus which is the site of ribosome production.

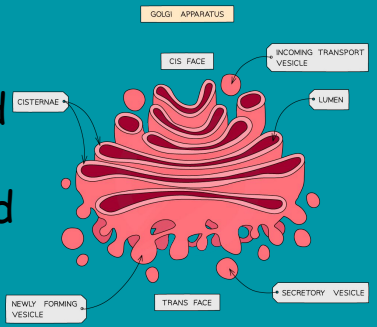
- **Rough endoplasmic reticulum** (RER) - a series of flattened sacs enclosed by a membrane with ribosomes on the surface. RER folds and processes proteins made on the ribosomes.

- **Smooth endoplasmic reticulum** - a system of membrane bound sacs. SER produces and processes lipids.

- **Golgi apparatus** - a series of fluid filled, flattened & curved sacs with vesicles surrounding the edges. Golgi apparatus processes and packages proteins and lipids.







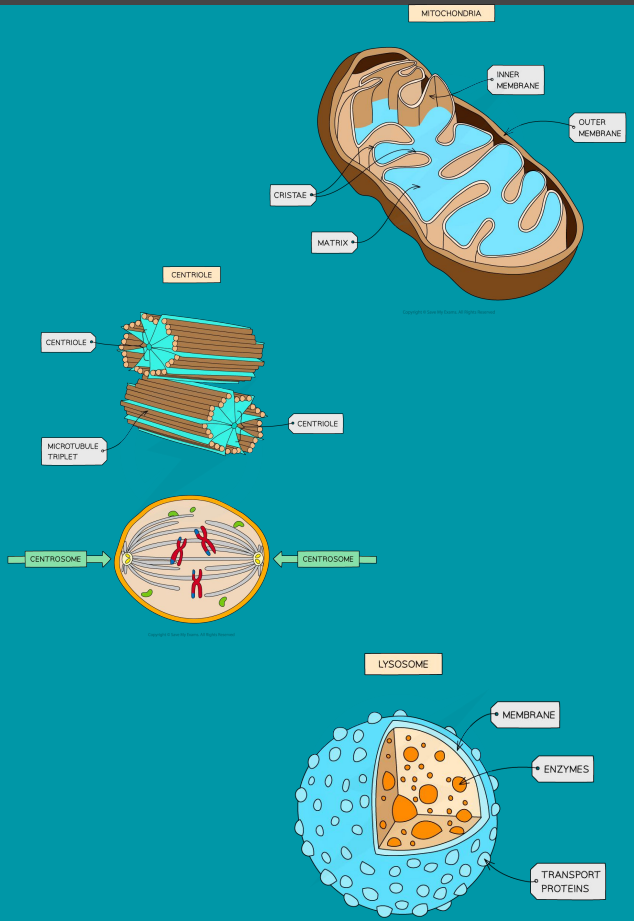
Cp 5

- **Mitochondria** usually oval shaped, bound by a double membrane called the envelope. The inner membrane is folded to form projections called cristae with a matrix on the inside, containing all the enzymes needed for respiration.

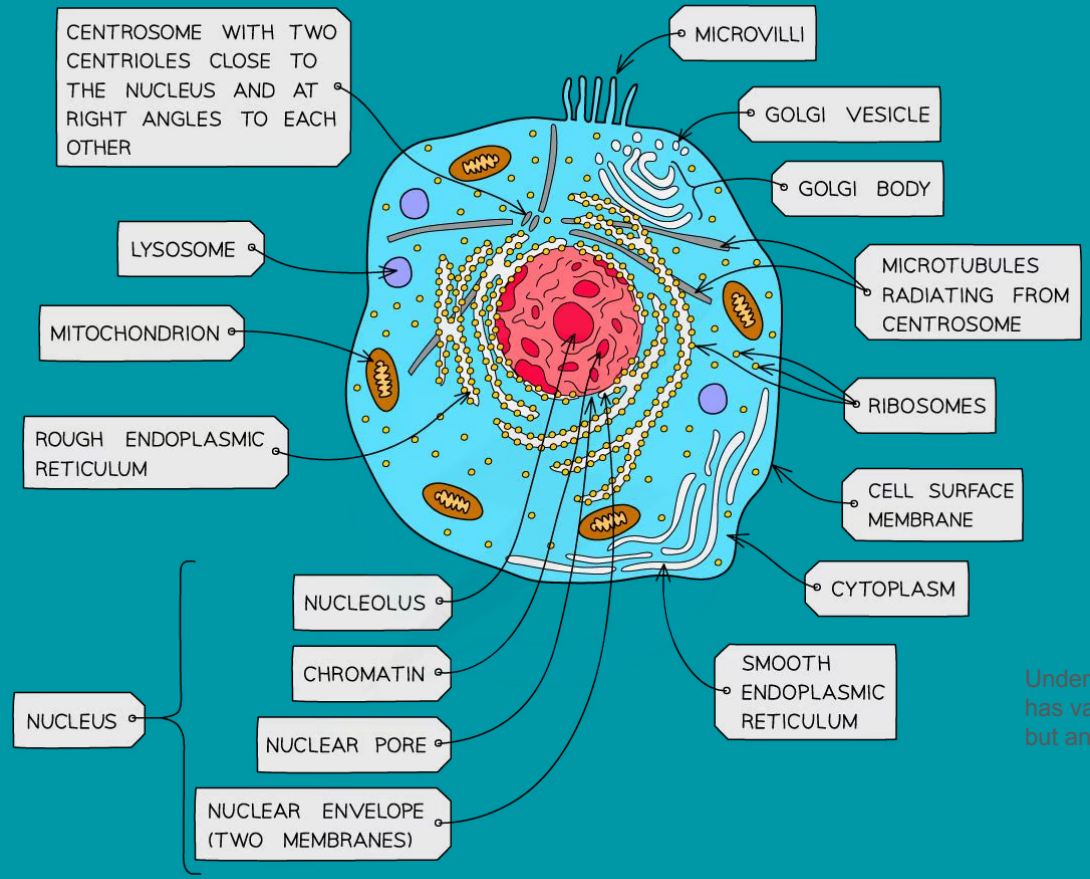
- **Centrioles** hollow cylinders ontaining a ring of microtubules rranged at right angles to each ther. Centrioles are involved in rell division.

- **Ribosomes**-these are composed of two subunits and are the site of protein production.

- **Lysosome**- a vesicle containing digestive enzymes bound by a single membrane.



Cp 5



Under light microscope :plant cell has vacuole, chloroplasts, cell wall but animal cell doesn't

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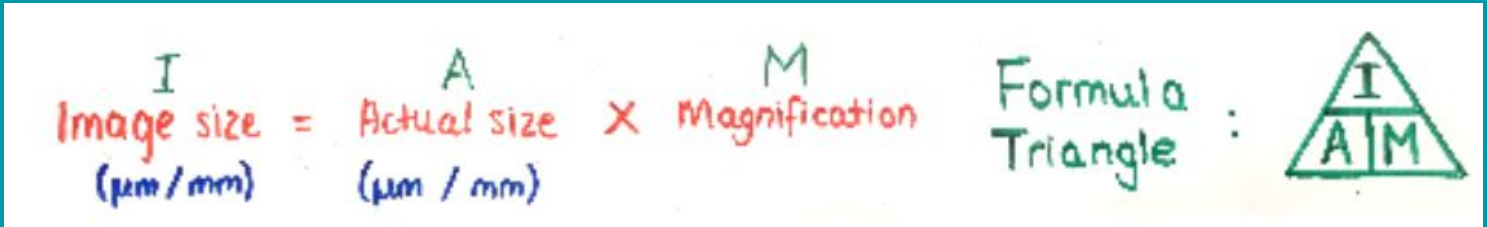
Cp 5

Calibration of eyepiece graticule:

How to accurately determine diameter of cell A? (5) oct 2023
1- use light microscope to find cell under low power then view under a high power
2- calibrate eyepiece graticule (Place a micrometer slide on the stage of the microscope Focus on the micrometer scale using the low power objective lens. Move the slide and rotate the eyepiece to align the scales of the eyepiece graticule and the micrometer scale in the field of view Count the number of divisions on the eyepiece graticule and compare them to a known length on the micrometer scale to figure out the length of one eyepiece unit Repeat steps 1-3 with the medium-power and high-power objective lens.)
3- count number of graticules units
4- convert eyepiece graticule units to microns using calibration
5- measure cell diameter at different positions



Cp 5

How to prepare a microscope slide:

1. For cheek - use cotton buds - gently rub on inside part pf ur cheeks - rub the cotton bud on a glass slide - add few drops of methylene blue - then cover with a cover slip

2. For plants - on a chopping board cut a thin transverse section of the plant tissue -thin section is needed to allow light to penetrate / pass through it . - mount with water to hold the sample on a slide - cover with a cover slip - remove excess water using absorbent

Safety -
obtaining cheek cells
cotton should be placed in a disinfectant - wash ur hands before and after - wear gloves - goggles - cotton buds should be used once for one individual

In general (preparation of a slide)
: - avoid skin contact with acetic orcin or methylene blue - safely use the microscope …using a light microscope with mirror (use daylight) , so don't place where sunlight might strike the mirror as this will damage the retina

Cp 5

Reasons for staining :
1. Add contrast to image.
2. To locate / see the position of particular type of chemical component.
3. To differentiate between different types chemicals/ components/ tissues (make it easier to identify type of cells or parts of cells under microscope).
4. For structures to be seen with more details

Over all uses of microscope
1. Cell and tissue identification.
2. Check for foreign organisms/ bodies.
3. Size , measurements of structures.

Errors: 1. In slide preparation: sample contamination , artefacts, wrong staining , dirty slides/lens.
2. In structure identification: leading to misdiagnosis.
3. Calculation errors or inability to use graticule.

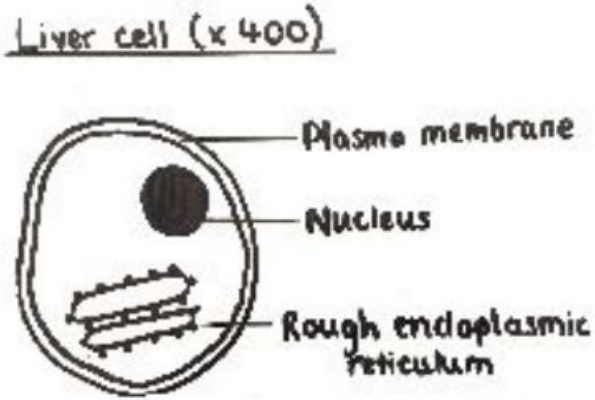
Stage micrometer is placed on stage of the microscope stage Allow to calibrate the eyepiece graticule Eyepiece graticule to allow to draw cells with correct proportions

Cp 5

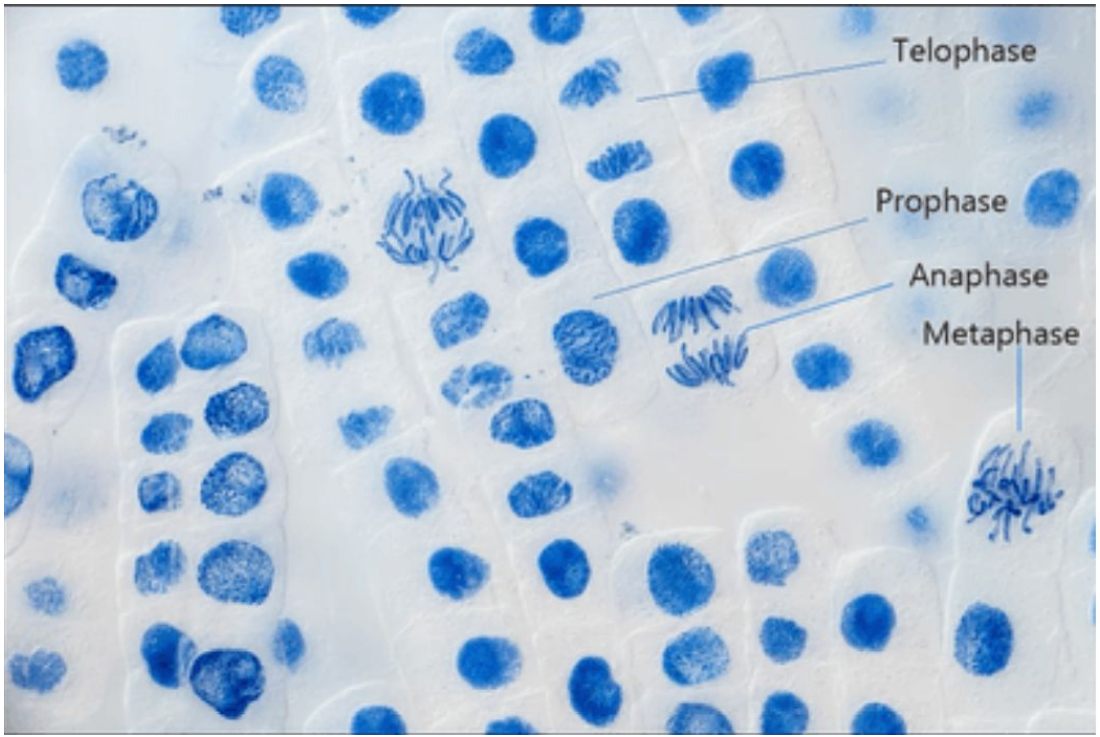
Biological drawings

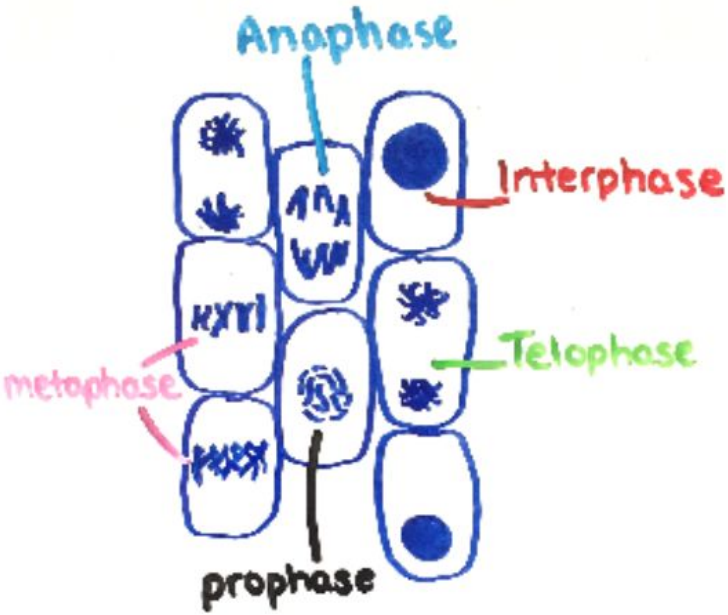
When producing biological drawings, in this case of cells being observed under the microscope, certain rules should be followed:

- Draw in pencil only
- Use blank white paper
- Do not sketch - draw clear, unfeathered lines only
- Include labels
- Label lines should be drawn with a ruler and not cross over each other
- Do not shade the drawing - for instance when indicating the thickness of membranes / cell walls instead draw 2 lines
- Include the magnification of the microscope in the title
- Make diagrams as simple as possible and draw them an appropriate size so everything can clearly be seen
- Include a scale bar where possible



Cp 6


Telophase
Prophase
Anaphase
Metaphase


Anaphase
Interphase
Telophase
metophase
prophase

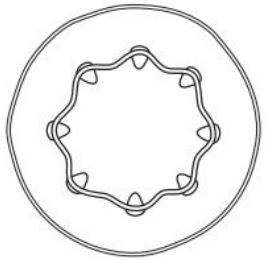
1 Animal and plant cells can be observed using a light microscope.

The structure of human cheek cells was investigated.

(a) Describe a safe method to prepare and examine the structure of human cheek cells.

Question Number	Answer	Additional Guidance	Mark
1(a)	An answer that includes four of the following points. • one reference to safety (1) and three of the following points • use of cotton bud (1) • followed by use of stain/dye (1) • place cells (on slide) under coverslip (1) • use of high power of microscope (1)	 e.g. bud into disinfectant/sterile/fresh bud/toothpick/wear gloves/ goggles/safe use of microscope/slides/careful use of bud/stain to prevent injury Accept toothpick/earbud/lollipop stick/glass rod/swab can piece together	(4)

(v) In another part of the investigation, a plan of the tissues in the stem was drawn.



Describe how a thin section of a stem could be prepared and viewed using a light microscope, in order to draw this plan.

(3)

1(a)(v)	An description that includes the following points: • description of cutting thin section (1) • use of {stain / dye} (1) • place section (on slide) under coverslip (1) • draw under low power / described (1)	e.g use of razor, botanical razor, sharp knife, scalpel, cutting transverse, horizontal ignore name NOT HP for drawing, ignore of they go on to HP so long as clear they would draw under LP.

Cp 6

1. Removal of the last 5-10 mm of root tips (meristem).
2. Place root tip in hydrochloric acid to separate the cells/macerate tissue/ soften tissue (making it easier to stain).
3. Addition of appropriate stain such as toluidine (blue) , acetic orcein, methylene blue to highlight chromosomes.
4. Place the root tip on microscope slide, teasing cells (using needle to spread cells), Squashing the cells underneath a cover slip to separate cells (for easier view), and heat the slide to intensify the stain.
5. Ensure the students view the preparation under the microscope on low power first to identify the area of dividing cells and to focus. Higher power will be required to examine the dividing cells for the stages of mitosis 6. Counting number of cells in mitosis, then counting total number of cells , then Calculate the mitotic index .

$$\text{Mitotic index} = \frac{\text{Cells in mitosis}}{\text{Total number of cells}} \times 100$$

Cp 6

1. Explain why the root tip is heated with acid. The root tip is heated with acid to break up the tissues into individual cells. The cellulose walls of plant cells are held together by pectins such as calcium pectate. Treatment with hydrochloric acid breaks this down. .

2. State the effect of maceration and pressing the slide preparation on the dividing cells. Maceration and pressing the preparation will separate the cells in the meristem tissue into individual cells in a single layer. This makes it easier to see the chromosomes and to identify the stages of division. .

3. Describe what information the cell counts give about each stage of mitosis. • The cell counts show the relative duration of each stage in the cell cycle. The longer a phase, the more cells are likely to be going through that phase at any point in time

4. Describe the role of mitosis in the life of an organism. Mitosis produces identical daughter cells for growth, replacement and repair

Risk assessment

Hazard	Risk	Precaution
Scalpel	Cuts from sharp edges	Take care when using it When carrying it around the lab place on a white tile Cut the root tip on a chopping board
Glassware	Cuts from sharp edges if broken	Have a dustpan and brush in the lab to sweep up and dispose of any broken glass immediately Avoid placing glassware on the edges of the lab benches Keep away from edge of desk
Hydrochloric acid and stain	Toxic if ingested or enters body; HCl is corrosive on skin.	Wear goggles and gloves when handling these Replace stoppers immediately after use Keep away from edge of desk

Determining the mitotic index of roots is one way to study growth of roots.

(i) Describe how the mitotic index of roots can be determined.

(4)

Number			
2(b)(i)	A description that includes 4 of the following points: • fixing root tip described (1) • stain used (1) • description of maceration / squashing (1) • view through high power microscope (1) • count of number of dividing cells and divide by total number of cells (1)	e.g. heating with conc. HCl Accept magnification quoted >/= 100x	(4)

Cp 6 mitosis
Q1. May/June 2017
Q2. Oct/Nov 2019 !!!!!!!! good q
Q2. May/June 2022

Cp 7

Observing for stem, root and leaf:
Place the specimen on a white tile. Wet the specimen, the tile and razor blade. Hold the specimen firmly and cut transverse sections sections of the specimen as thinly as possible. Use tweezers to pick them up carefully and place on a microscope slide. Add a few drops of the stain Toluidine blue. Place a coverslip on it gently. View the specimen under a microscope in low power. Bring the lens as close to the slide as possible. Using the coarse adjustment on the microscope, slowly move away from the slide. After, use the fine adjustment to focus until cells are clearly visible. Sketch a plan diagram of the plane of view using a pen and paper and label each part.

Safety: 1. Wear eye protection. 2. Avoid all skin contact with the toluidine blue stain; disposable gloves may be worn. 3. Take care when using razor blades and mounted needles

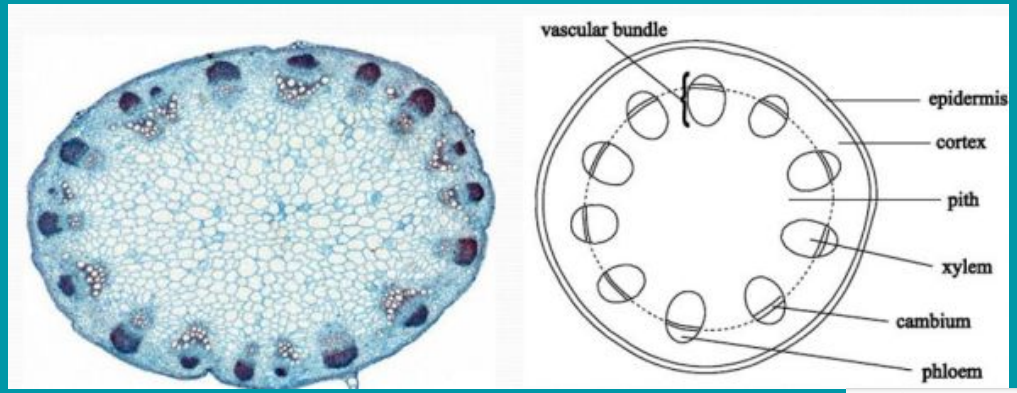
Stains
1. Toluidine blue O is a dye. It is a metachromatic stain and interacts with different chemical components of cells to produce a variety of colours. • It can help us distinguish between phloem and xylem tissue as it stains the pectins in primary cell walls of phloem cells pinkish purple and stains the lignin and tannins of secondary cell walls, found in xylem vessels, blue or blue-green. • This is an example of differential staining – different types of cells are stained differently. This stain also shows up nuclei, plastids such as chloroplasts, and vacuoles.blindness. 2. Acetic orcine, hematoxylin, acetocarmine, methyl blue. • These stains absorb more light making it easier to see the structures. • And allow making the transparent structures visible.

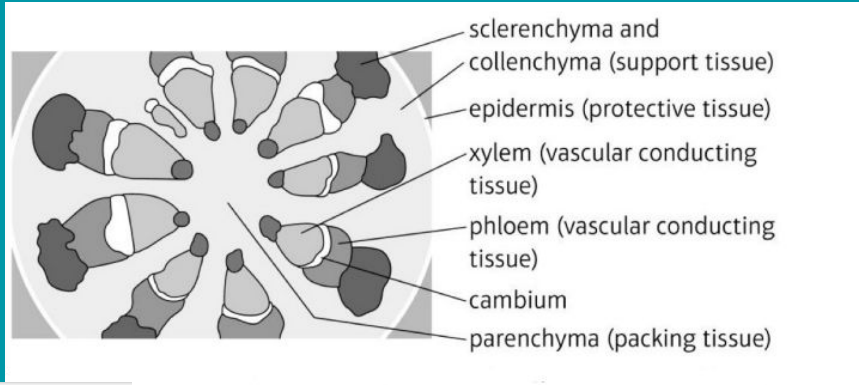
Cp 7

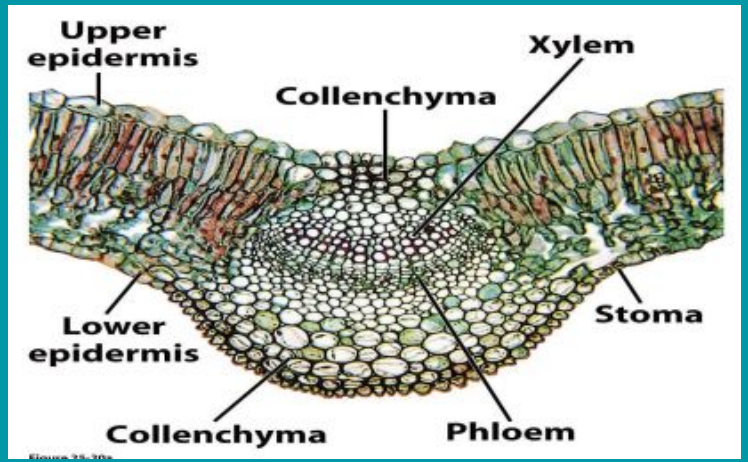
Risk assessment

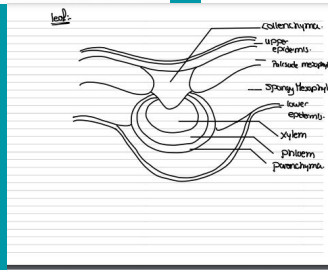
Risk	Hazard	Precaution
Toluidine blue O stain	Could cause an allergic reaction, may irritate the skin if contact occurs	Use eye protection and disposable gloves when handling If it comes into contact with skin then rinse with cold, running water
Stem, root and leaf	Could cause an allergic reaction	Minimise contact, wear gloves if an allergy is present
Razor	Cuts from sharp objects	Take care when using; keep away from the edge of the desk Carry it on a white tile when moving around the lab Keep away from edge of desk
Microscope	Heavy object	Do not use on the edge of the desk as it could be knocked onto body parts such as the feet

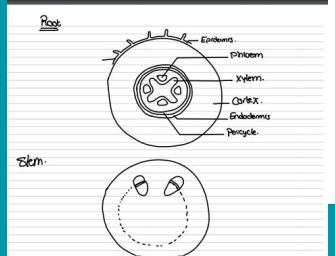
Cp 7


vascular bundle
epidermis
cortex
pith
xylem
cambium
phloem


sclerenchyma and collenchyma (support tissue)
epidermis (protective tissue)
xylem (vascular conducting tissue)
phloem (vascular conducting tissue)
cambium
parenchyma (packing tissue)


Upper epidermis
Xylem
Collenchyma
Lower epidermis
Stoma
Collenchyma
Phloem


leaf.
collenchyma.
upper epidermis.
Palisade mesophyll
Spongy Mesophyll
lower epidermis.
Xylem
Phloem
Parenchyma.


Root.
Epidermis.
Phloem
Xylem.
Cortex.
Endodermis
Pericycle.
Stem.

Cp 7

1. Suggest why a stain was used on the stem and root cells.? The thin sections of stem and root do not absorb much light, so they are difficult to see using a light microscope. The stain absorbs more light, making it easier to see the structures. Not all of the cells are stained so differences can be seen.

2. Explain why it was important not to use too much stain when preparing the slide. If too much stain is used, it will be difficult to see anything using the microscope. Any differences between the cells will be hidden as the stain will absorb all the light.

3. Describe a method for comparing the size of the xylem tissue in the root and stem samples.
• By using a micrometer slide and an eyepiece graticule. The graticule is calibrated using the micrometer slide. The size of the xylem tissue can then be calculated and compared.

Cp 7

(b) The movement of substances in the phloem is called translocation.

I)Describe how the companion cells in phloem contribute to translocation.(2 marks)

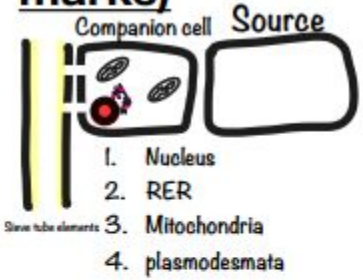
Translocation in the phloem happens through tubes connected in chains called the sievetube element. The sieve-tube element is next to the companion cell. The companion cells are connected to the sieve-tube element with plasmodesmata, which allows materials to flow between the cells. (1) The sieve-tube elements are alive, yet they lack organelles such as nuclei or ribosomes. The organelles of the companion cells carry out all the metabolic functions of the sieve-tube element. Without the companion cells, translocation would not be possible because the sieve-tube element would die.

(b) ii) . Explain how the companion cells are adapted to their function..(2 marks)

The companion cell is able to carry out translocation because it usually has a larger number of ribosomes and mitochondria than a typical plant cell. This makes the companion cell more metabolically active than a 'typical' plant cell and it is therefore able to carry out translocation. (1) The cytoplasm of a companion cell is connected to the sieve-tube element by plasmodesmata. This allows materials to flow between the companion cell and the sieve tube

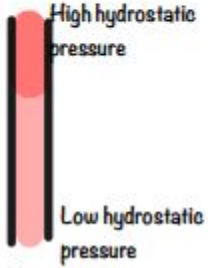
Cp 7

marks)



Sucrose move nto comapnion cells against its gradient

Xylem	Phloem
transports water and minerals	*transports products of photosynthesis* **(1)**
dead tissue **(1)**	living tissue
transports up and down the stem **(1)**	transport up or down the stem
transport as mass flow	*transports as mass flow* **(1)**
transport depends on pull from transpiration	*transport depends on ATP from respiration* **(1)** + hydrostatic pressure gradient

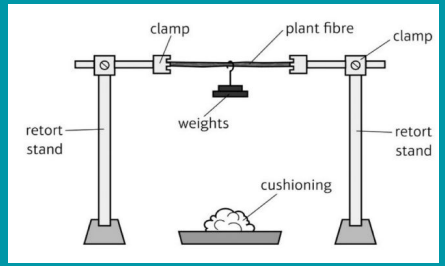


Cp 8

Tensile strength is the maximum amount of force a fiber can take before failing/breaking. Affected by Length of fibre Humidity of fibre Cross-sectional Area Type of plant fibre.

Effect of lengths on tensile strength

1. Get fibres from same plant , of same age and same diameter .
2. But of different lengths …….carry the experiment under same temperature , humidity , and thickness (diameter) .
3. clamp one of the 20cm string between two retort stands
4. Add Cushion underneath the string as as not to hurt your feet / to avoid hitting the bench when string breaks
5. Gradually add increasing masses until the fibre breaks .
6. Record the mass of which the fibre breaks
7. Convert mass of which the fibre breaks Tensile strength = breaking force / cross sectional area
8. Repeat same steps with other fibres of other lengths .



Improve accuracy : smaller mass intervals
Improve reliability : Repeat 3 more times at each length Calculate average value (SD and plot a graph)

Cp 8 tensile strength

2 In an investigation, the tensile strength of fibres from five plant species was determined.

The breaking force was measured by finding the mass in grams that would break the fibre.

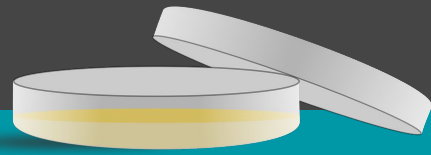
(a) Describe a valid method that could be used to compare the breaking force of these fibres.

(4)

Question Number	Answer	Additional Guidance	Mark
2(a)	A description that includes the following points. • how fibre fixed (1) • how force applied (1) • one variable which should remain constant stated (1) • replicate (the experiment/measurements) (1) • (and) calculate the mean/SD (1)	'attached to a retort stand / hanging from a clamp' e.g. gradual addition of masses / increase tension of a newton metre gradually e.g. Relative humidity / temperature / length of fibre	**(4)**

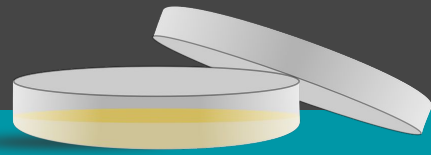
Cp 8 tensile strength
Q1. Jan 2016
Q2. Jan 2022
Q1. May/June 2023

Cp 9



1. Test for different parts of the plant/ different plants
2. Grinding plant materials to make extract
3. Prepare a lawn of bacteria .
4. Allow aseptic technique : using bunsen burner at yellow flame to creat a convection current to carry a way air borne bacteria , sterile the inoculating loop and the neck of flask containing bacterial lawn .
5. On agar petri dish . Distribute bacteria evenly using the streaking method
6. Create 4 equal sized wells (opening in the gel) .
7. Place the paper discs soaked with different plant extracts . ….and the fourth paper disc soaked with water as a control experiment .
8. Seal the lid at both sides but not all way round with cello-tape, to prevent anaerobic conditions that might kill the bacteria in culture and cause growth growth of anaerobic bacteria
9. Incubate at temperature 20-35 (27 C) for 24 hours (to 7 days) to prevent the growth of pathogenic organisms.
10. Measure the diameter of zone of inhibition
11. Then disposal ..putting petri dish in a plastic bag , seal and sterile at 121 C for 15 mins under high pressure and temperature then throw it away .
12. Repeat and calculate the mean value

Cp 9



Aseptic technique :
1. Keep bunsen burner on yellow flame on bench
To create convection current to carry away airborne bacteria
2. Sterile tools such as the inoculating loop and the neck of the flask containing the bacterial sample by
heating them in bunsen burner
3. Always when opening the lid of petri dish containing bacteria ....allow slight degree opening to avoid
contamination with another microorganisms causing disease .
4. Fastening to the lid using cello-tape, but dont tape all way round to avoid anaerobic conditions

Cp 9 antimicrobial properties of plants

1 Some plant extracts have been shown to have antimicrobial properties.

(a) Describe a method that can be used to study the antimicrobial properties of a plant extract.

(5)

Number			
1a	An answer that includes five of the following points. • description of {bacteria grown (on nutrient agar) / bacterial lawn} / bacterial broth (1) • filter paper discs soaked in plant extract (placed on agar) / plant extract placed in well (cut in agar) / plant extract added to broth (1) • control variable described (1) • one safety aspect described (1) • incubate for suitable time (1) • clear zone around {disc / well} {measured / scored / turbidity measured (1)	Accept microbe e.g. disc size / extract volume / temperature e.g. flaming / dish lid / use autoclave / safe temperature quoted (<32 C) Accept 24 hours to 1 week Accept cloudiness	(5)

Cp 9 antimicrobial properties of plants
Q3. Oct/Nov 2019
Q1. May/June 2021
Q1. Jan 2022
Q3. May/June 2023

Additional cps

RECOMMENDED ADDITIONAL PRACTICAL

- Investigate the structure of a mammalian heart by dissection.
- Investigate tissue water potentials using plant tissue and graded concentrations of a solute
- Use a semi-quantitative method to estimate protein concentration using biuret reagent and colour standards.
- Investigate factors affecting the growth of pollen tubes.

Q3. May/June 2021
Q1. May/June 2022
Q2. May/June 2023

- Investigate plant mineral deficiencies.

Q3. May/June 2022
Q2. Jan 2021
Q1. May/June 2015
Q1. Oct/Nov 2019

- AL : Investigate habituation to a stimulus.

?? (idk what category this goes into) Q1. Oct/Nov 2018

Sources:
Znotes u3 notes
Pmt u3 notes
Nihal gabr notes
IAL past papers
Dynamic papers